MEWBURN ELLIS
INTELLECTUAL PROPERTY
PATENTS · TRADE MARKS · DESIGNS

Chartered Patent Agents
European Patent Attorneys
European Trade Mark Attorneys
Trade Mark Agents

York House
23 Kingsway
London WC2B 6HP

Tel 020 7240 4405
Fax 020 7240 9339
Fax 020 7240 0652 (G4)
www.mewburn.com
mail@mewburn.com

**Partners**
David Harrison
Ian Armitage
Hugh Paget
Michael Ford
Roger Calderbank
Patrick Stoker
Seán Walton
Nigel Hackney
Simon Kiddle
Adrian Brasnett
Roger Grimshaw*
Simon Kremer
Joanna Cripps
Robert Watson

*not CPA or EPA

**Assisted by**
Sofia Arenal
Ray Coleiro
Chris Denison
Michael Foster
Michael Freeman
Stephen Gill
Susan Harrison
Sheila Hill
Allan Hoyano
June Lyons
Mary Medycky
Matthew Naylor
Kathryn Nichols
Alastair Rawlence
Michael Sanderson
Ian Stuart
Nicholas Sutcliffe
David Taylor
Rachel White

European Patent Office
PB 5818 Patentlaan 2
2280 HV Rijswijk (ZH)
NETHERLANDS

20 September 2002

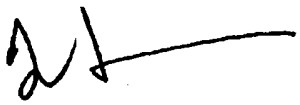
EPO - Munich
73
24. Sep. 2002

Dear Sirs

**European Patent Application No: 01945653.2**
**Applicant: Rohto Pharmaceutical Co., Ltd.**
**Our Ref: IS/FP5996236**

I file herewith a translation of Japanese patent application no. 2000-195804 from which the European application claims priority.

Yours faithfully



Ian Stuart
Authorised Representative
MEWBURN ELLIS
ian.stuart@mewburn.com

(To contact me personally
please phone +44 117 2966411)

Enc. Translation of priority document

/pae

PATENT OFFICE

JAPANESE GOVERNMENT

This is to certify that the annexed is a true copy of the following application as filed with this Office.

Date of Application: June 29, 2000

Application Number: Patent Application No. 2000-195804

Applicant: Rohto Pharmaceutical Co., Ltd.

Document name: Application for patent

Docket No.: DR01J162

Date of Application: June 29, 2000

Addressee: Commissioner, Patent Office

International Patent Classification: A61K 33/00

A61K 47/26

A61K 47/30

A61J 1/00

Inventor:

Address: c/o Rohto Pharmaceutical Co., Ltd.
8-1, Tatsuminishi 1-chome,
Ikuno-ku, Osaka-shi, Osaka-fu

Name: Toru NISHIHARA

Inventor:

Address: c/o Rohto Pharmaceutical Co., Ltd.
8-1, Tatsuminishi 1-chome,
Ikuno-ku, Osaka-shi, Osaka-fu

Name: Yoshiomi SOEJIMA

Inventor:

Address: c/o Rohto Pharmaceutical Co., Ltd.
8-1, Tatsuminishi 1-chome,
Ikuno-ku, Osaka-shi, Osaka-fu

Name: Toshiki KURUMADA

Applicant:

Identification No.: 000115991

Name: Rohto Pharmaceutical Co., Ltd.

Agent:

Identification No.: 100077012

Patent Attorney:

| | |
|---|---|
| Name: | Ryo IWATANI |
| Phone No.: | 06-4796-1300 |

Payment of fees:

| | |
|---|---|
| Prepayment Book No.: | 066372 |
| Amount to be paid: | ¥21,000 |

Attached document:

| | | |
|---|---|---|
| Item: | Specification | 1 copy |
| Item: | Drawings | 1 copy |
| Item: | Abstract | 1 copy |

Request for proof transmission: Yes

Document name: Specification

Title of the invention: OXYGEN-CONTAIINNG OPTHALMIC COMPOSITION

What is claimed is:

1. An ophthalmic composition, which is enriched with oxygen.

2. The ophthalmic composition according to claim 1, wherein the composition comprises oxygen and/or an oxygen donor, and the amount of oxygen present in the composition is 10 mg or above per liter of the composition.

3. The ophthalmic composition according to claim 1, wherein the composition comprises oxygen and/or an oxygen donor, and the amount of oxygen present in the composition is 8.84 mg/l or above per liter of the composition at 1 atmospheric pressure, 20°C.

4. The ophthalmic composition according to claim 1, wherein the content of oxygen is at least 95% of the saturated dissolved oxygen in the composition in the presence of air at 1 atmospheric pressure.

5. The ophthalmic composition according to any one of claims 1 to 4, which is selected from the group consisting of eye drop, eyewash and contact re-wetting and/or wetting solution.

6. The ophthalmic composition according to any one of claims 1 to 5, wherein the oxygen donor is an oxygen carrier comprising captured oxygen.

7. The ophthalmic composition according to claim 6, wherein the oxygen carrier is fluorocarbon.

8. The ophthalmic composition according to claim 6, wherein

the oxygen carrier is one or more substances selected from the group consisting (a) hemoglobin or a polymer thereof; (b) modified hemoglobin or a polymer thereof, (c) porphyrin complex or a polymer thereof; and (d) a lipid endoplasmic reticulum which includes hemoglobin or a polymer thereof; modified hemoglobin or a polymer thereof; or porphyrin complex or a polymer thereof.

9. The ophthalmic composition according to any one of claims 1 to 8, which further comprises at least one substance selected from the group consisting of inorganic salts, sugars, vitamins, amino acids, epithelial cell growth factors and active oxygen scavengers.

10. The ophthalmic composition according to any one of claims 1 to 9, which is stored in a container impermeable or less permeable to oxygen.

11. The ophthalmic composition according to any one of claims 1 to 9, which is stored in an oxygen-permeable container that is encased in a package impermeable or less permeable to oxygen.

12. The ophthalmic composition according to claim 11, wherein the room formed between the container and the package is replaced with oxygen.

13. The ophthalmic composition according to any one of claims 10 to 12, wherein the container impermeable or less permeable to oxygen or the package impermeable or less permeable to oxygen comprises ethylene-vinyl alcohol copolymer.

14. The ophthalmic composition according to any one of claims 10 to 13, which is packaged in a unit dosage container.

15. The ophthalmic composition according to any one of claims 1 to 14, which is useful for preventing or treating keratitis superficialis punctate, corneal vascularization, corneal epithelial erosion, acute corneal epithelial edemas, corneal infiltration or corneal endotherial disorders.

16. The oxygen carrier according to claim 7 or 8, which is useful for producing the ophthalmic composition according to claim 1.

Detailed explanation of the invention

[0001]

Technical field to which the invention pertains:

The present invention relates to an ophthalmic composition enriched with oxygen.

[0002]

Prior art:

The majority of tissues and organs of a living body are supplied with oxygen, however, in the case of cornea, oxygen cannot be supplied to the cornea through blood. Therefore, it is necessary to supply oxygen to the cornea by a certain external means in order to keep the cornea healthy.

Unless a sufficient amount of oxygen does not reach the cornea, corneal edema (corneal swelling) first breaks out. If the cornea is deficient in oxygen for a long time, corneal disorder, such as vascular infiltration harmful in the cornea, is manifested. Furthermore, corneal disease such as keratitis superficialis punctate, corneal vascularization, corneal epithelial erosion, acute corneal epithelial edema, corneal

infiltration or corneal endotherial disorders is also caused.

[0003]

Oxygen is present in tear in a dissolved form. Usually, the cornea uptakes oxygen from the dissolved oxygen in tear. When the tear volume decreases, the amount of oxygen to be supplied to the cornea decreases, and thus the above disorders are liable to occur. Examples of the decrease in the amount of oxygen in tear include cases of dry eye, dry eye due to environment, or contact lenses wearers. In the cases as above, corneal disorder due to deficient supply of oxygen is often found.

[0004]

Dry eye is a disease wherein the surfaces of eyes are disordered due to a reduction in the amount of tear or a change in the quality of tear. Since a dry eye patient's tear is not in normal condition, the surfaces of eyes cannot sufficiently be protected with tear. As a result, the cornea is deficient in oxygen.

Furthermore, dry eye or tear deficiency due to aging, work that overtasks eyes (a computer, a word processor, driving, minute work, reading and so on) causes oxygen deficiency in the cornea.

[0005]

Contact lenses are classified into two types by their material, i.e., a hard contact lens and a soft contact lens. A hard contact lens can further be classified into a PMMA (polymethylmethacrylate) lens showing non-oxygen-

permeability and a lens showing improved oxygen-permeability (RGP lens, $O_2$ lens) in view of oxygen-permeability.

A hard contact lens does not absorb water or does not allow water to pass, either. However, since the hard contact lens does not cover the whole cornea, oxygen is supplied to the cornea by tear that is exchanged at the border of the lens.

A PMMA lens, however, shows no-oxygen-permeability, and thus the supply of oxygen is deficient. In order to offset this drawback, a reduction in the size of a lens, improvement in design, and so on have been carried out. However, there has not been offered yet a contact lens that can prevent the above-mentioned problems completely.

Furthermore, lens materials have been changed to those having high-oxygen permeability, and thus oxygen is supplied to tear through a hard contact lens, but the amount of oxygen supplied to the cornea has not reached a satisfactory level compared with that of a naked eye.

[0006]

On the other hand, in the case of a soft contact lens, which is typically manufactured into a form that fits well to the ocular surface and has a size sufficient to cover the whole cornea. Accordingly, the tear exchange rate at the border of the lens is lower. In addition, oxygen is supplied to the cornea only through tear. When the tear volume decreases accompanied by evaporation of water from the lens surface, the amount of oxygen supplied to the cornea decreases.

Further, it is reported that not the whole water present at a soft contact lens can move, and only a part thereof can move. As described above, water seldom moves inside a soft contact lens, and between the soft contact lens and the cornea, and hence it takes time to supplement oxygen that corneal cells have consumed. Therefore, the cornea continues to be in a oxygen deficient condition.

Furthermore, even if the amount of water contained in the lens increases, a sufficient amount of oxygen cannot be supplied to the cornea and thus corneal disorder such as corneal edema or vascular infiltration cannot be avoided while wearing the lens for a long period of time.

Accordingly, particularly in the soft contact lens, oxygen supply to the cornea becomes a problem.

[0007]

As mentioned above, the necessity of supplying oxygen to the cornea is understood. As means of increasing the amount of oxygen supply, methods for temporary improvement in tear volume with the use of eye drops or artificial tears have been taken, though these methods are aiding means of supplementing tear from the outside.

However, in these methods, oxygen dissolved in eye drops or artificial tears at normal pressure is merely supplied to the cornea, and thus positive oxygen supply such as supplying oxygen at a high concentration is not performed. In conventional eye drops or artificial tears, the amount of dissolved oxygen in a composition is small, and thus the

diffusion rate of oxygen in tear is slow. Further, the oxygen uptake rate to the cornea is also slow.

Incidentally, the concentration of oxygen in water saturated with dissolved oxygen at 20°C in the presence of air of 1 atmospheric pressure is about 8.84 mg/L. However, the amount of dissolved oxygen contained in eye drops or water commercially available is 10-20 % less than that of saturated dissolved oxygen concentration.

From the above, the development of an ophthalmic composition enriched with oxygen was desired in order to prevent, alleviate or treat symptoms due to oxygen deficiency in the cornea.

[0008]

Furthermore, an ophthalmic composition enriched with oxygen has a problem of preservation. That is, various containers made from Teflon, polystyrene, polyethylene or polypropylene, which have been used as containers for eye drops, are all oxygen-permeable. Thus, when an ophthalmic composition enriched with oxygen is charged in them, it is not possible to prevent oxygen from evaporating in the air with time. For that reason, when such containers are utilized, there was a drawback in that a reduction in the amount of dissolved oxygen in an ophthalmic composition with time could not be avoided.

[0009]

Problem to be solved by the invention:

An object of the present invention is to provide an ophthalmic composition enriched with oxygen.

Another object of the present invention is to provide an oxygen-rich ophthalmic composition containing an oxygen carrier comprising high-concentration oxygen or captured oxygen.

Another object of the present invention is to provide an oxygen-rich ophthalmic composition filled in a container or encased in a package, which enables preservation of the composition without reducing the dissolved oxygen amount, followed by sealing up.

Another object of the present invention is to provide an oxygen-rich ophthalmic composition for prevention, alleviation or treatment of corneal disorder, which can supply oxygen to the cornea.

[0010]

Means of solving the problem:

In order to solve the above problem, the present inventors intensively studied and found that symptoms due to oxygen deficiency in the cornea could be prevented, alleviated or treated by an oxygen-rich ophthalmic composition, namely, an ophthalmic composition, wherein the content of oxygen is at least 95% of the saturated dissolved oxygen in the presence of air at 1 atmospheric pressure, or an ophthalmic composition, wherein the composition comprises oxygen and/or an oxygen donor, and the amount of oxygen present in the composition is 10 mg or above per liter of the composition.

The term "oxygen donor" refers to a substance wherein oxygen is captured by a carrier. The term "oxygen

carrier" refers to a compound having ability (capacity) to capture and transport oxygen. Examples of preferred carrier include those which capture oxygen under high oxygen partial pressure and discharge oxygen under low oxygen partial pressure.

[0011]

The present inventors also found that, by dissolving oxygen at a low temperature and/or under high oxygen partial pressure, an oxygen-rich opthalmic composition could easily be prepared.

Further, an oxygen-rich opthalmic composition can also be prepared by using an oxygen carrier. In this case, examples of the oxygen carrier usable include (a) fluorocarbon, (b) hemoglobin or a polymer thereof, (c) modified hemoglobin or a polymer thereof, (d) porphyrin complex or a polymer thereof; and (e) a lipid endoplasmic reticulum, which includes hemoglobin or a polymer thereof, modified hemoglobin or a polymer thereof, or porphyrin complex or a polymer thereof.

[0012]

It was also found that, by using a container impermeable or less permeable to oxygen as a container for an oxygen-rich opthalmic composition, or by using a container permeable to oxygen as a container for the ophthalmic composition and encasing the composition-filled container in a package impermeable or less permeable to oxygen followed by sealing up, the ophthalmic composition could be preserved without reducing the dissolved oxygen amount in the ophthalmic

composition.

Furthermore, it was also found as follows: an oxygen-rich ophthalmic composition or an ophthalmic composition prepared in accordance with a conventional method was filled in a container permeable to oxygen. Then this container was encased in a package impermeable or less permeable to oxygen followed by sealing up. Together with this, a void space formed between the container and the package was replaced with oxygen gas, whereby an oxygen-rich ophthalmic composition could be prepared and preserved.

The present inventors further studied and have attained the present invention.

[0013]

That is, the present invention relates to

(1) An ophthalmic composition, which is enriched with oxygen;

(2) The ophthalmic composition set forth in (1), wherein the composition comprises oxygen and/or an oxygen donor, and the amount of oxygen present in the composition is 10 mg or above per liter of the composition;

(3) The ophthalmic composition set forth in (1), wherein the composition comprises oxygen and/or an oxygen donor, and the amount of oxygen present in the composition is 8.84 mg/l or above per liter of the composition at 1 atmospheric pressure, 20°C;

(4) The ophthalmic composition set forth in (1), wherein the content of oxygen is at least 95% of the saturated dissolved oxygen in the composition in the presence of air at 1 atmospheric pressure;

(5) The ophthalmic composition set forth in any one of (1) to (4), which is selected from the group consisting of eye drop, eyewash and contact re-wetting and/or wetting solution;

(6) The ophthalmic composition set forth in any one of (1) to (5), wherein the oxygen donor is an oxygen carrier comprising captured oxygen;

(7) The ophthalmic composition set forth in (6), wherein the oxygen carrier is fluorocarbon;

(8) The ophthalmic composition set forth in (6), wherein the oxygen carrier is one or more substances selected from the group consisting (a) hemoglobin or a polymer thereof; (b) modified hemoglobin or a polymer thereof, (c) porphyrin complex or a polymer thereof; and (d) a lipid endoplasmic reticulum which includes hemoglobin or a polymer thereof; modified hemoglobin or a polymer thereof; or porphyrin complex or a polymer thereof;

(9) The ophthalmic composition set forth in any one of (1) to (8), which further comprises at least one substance selected from the group consisting of inorganic salts, sugars, vitamins, amino acids, epithelial cell growth factors and active oxygen scavengers;

(10) The ophthalmic composition set forth in any one of (1) to (9), which is stored in a container impermeable or less permeable to oxygen;

(11) The ophthalmic composition set forth in any one of (1) to (10), which is stored in an oxygen-permeable container that is encased in a package impermeable or less permeable to oxygen;

(12) The ophthalmic composition set forth in (11), wherein the

room formed between the container and the package is replaced with oxygen;

(13) The ophthalmic composition set forth in any one of (10) to (12), wherein the container impermeable or less permeable to oxygen or the package impermeable or less permeable to oxygen comprises ethylene-vinyl alcohol copolymer;

(14) The ophthalmic composition set forth in any one of (10) to (13), which is packaged in a unit dosage container;

(15) The ophthalmic composition set forth in any one of (1) to (14), which is useful for preventing or treating keratitis superficialis punctate, corneal vascularization, corneal epithelial erosion, acute corneal epithelial edemas, corneal infiltration or corneal endotherial disorders; and

(16) The oxygen carrier set forth in (7) or (8), which is useful for producing the ophthalmic composition set forth in (1).

[0014]

Mode for carrying out the invention:

"Oxygen-rich opthalmic composition" in the present invention means an ophthalmic composition wherein the content of oxygen in the composition is positively (artificially) increased. Thus, it means that the ophthalmic composition of the present invention contains more (excessive) oxygen than that normally dissolved under the same conditions. The ordinary temperature is between about 15 ℃ and 25 ℃, and the ordinary pressure is around 1 atmospheric pressure (1 atm). The oxygen concentration of the ophthalmic composition of the present invention is preferably at least 95%, more preferably

equal or above of the saturated dissolved oxygen in the presence of air at 1 atmospheric pressure.

The oxygen content of the present composition containing oxygen and/or oxygen donor is about 8.84 mg or above, preferably about 8.84 - 225 mg, more preferably about 10 - 220 mg, further more preferably about 14 - 180 mg, per liter of the composition.

Incidentally, the ophthalmic composition of the present invention is usually an aqueous solution, and an aqueous solution, suspension or emulsion is preferred. An emulsion is particularly preferred.

[0015]

The oxygen-rich ophthalmic composition of the present invention can be prepared by any methods used for making a composition containing an excessive amount of oxygen. However, it can be conveniently and preferably prepared by adding oxygen donor, which is consisting of a carrier and oxygen captured, or dissolving excess oxygen gas into a composition.

[0016]

As an oxygen-rich ophthalmic composition, an ophthalmic composition containing an oxygen donor wherein an oxygen carrier has captured oxygen is included.

Examples of the oxygen carrier include (a) fluorocarbon, (b) hemoglobin or a polymer thereof, (c) modified hemoglobin or a polymer thereof, (d) porphyrin complex or a polymer thereof; and (e) a lipid endoplasmic reticulum, which includes hemoglobin or a polymer thereof, modified hemoglobin

or a polymer thereof, or porphyrin complex or a polymer thereof. They may be used alone or in combination of two or more.

[0017]

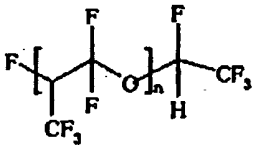
Fluorocarbons to be used as oxygen carriers in the present invention are derived from organic compounds wherein a part or all of hydrogen atoms are replaced with fluorine. Preferable fluorocarbons are those which have a capacity to transport oxygen and are physiologically acceptable.

Fluorocarbon is a physiologically acceptable substance because it is excreted from the lung with expiration without undergoing metabolization, even if absorbed into a body.

[0018]

Examples of fluorocarbon usable as an oxygen carrier in the present invention include perfluorobutyltetrahydrofuran ($C_8F_{16}O$), which can be represented by the formula (1):

[Chemical formula 1]



wherein n is 4, perfluorinate ether, perfluorocarbon or perfluoro-tert-amine. They may be used in combination of two or more compounds.

[0019]

Examples of perfluorocarbon include

perfluorocycloalkane, perfluoroalkylcycloalkane, or perfluoroalkane. The carbon number in these compounds is preferably 5 - 20, more preferably 9 - 11.

Perfluoroalkylcycloalkane may contain a hetero ring in place of cycloalkane. Examples of perfluoroalkylcycloalkane include perfluoro-$C_{3-5}$-alkylcyclohexane such as perfluoromethylpropylcyclohexane, per-fluorobutylcyclohexane, perfluorotrimethylcyclohexane, perfluoroethyl-propylcyclohexane, perfluorodecalin or perfluoromethyldecalin; per-fluoro-$C_{4-6}$-alkyltetrahydropyran such as perfluorohexyl-tetrahydro-pyran; perfluoro-$C_{5-7}$-alkyltetrahydrofuran such as perfluoro-pentyl-tetrahydrofuran, perfluorohexyltetrahydrofuran, or perfluoro-heptyl-tetrahydrofuran.

[0020]

Examples of perfluoro-tert-amine include perfluoro-tert-alkylamine, perfluoro-N-$C_{4-6}$-alkylpiperidine and perfluoro-N-$C_{5-7}$-alkyl-morpholine. The carbon number in these compounds is preferably 3 - 20, more preferably 9 - 11.

Examples of perfluoro-tert-alkylamine include perfluoro-trialkyl-amine such as perfluoro-N, N-dibutylmonomethylamine, perfluoro-N, N-diethylpentylamine, perfluoro-N, N-diethylhexylamine, perfluoro-N, N-dipropyl butyl amine, perfluorotripropylamine, or the like; perfluoro-N,N-dialkylcycloalkylamine such as perfluoro-N,N-diethylcyclohexyl-amine, or the like.

[0021]

Preferably, a fluorocarbon can be used as an oxygen carrier in the present invention as an emulsion which is stable in physiologically acceptable aqueous medium or is prepared on the occasion of use by suspending the fluorocarbon into an aqueous solvent.

For example, an emulsion can be prepared by mixing a fluorocarbon, an emulsifier, optionally an emulsification auxiliary agent, further optionally a solubilizing agent such as salts, alcohols or propylene glycol, a stabilizer, a buffer, water and a pH adjusting agent in a physiologically acceptable aqueous solvent such as distilled water, physiological saline or PBS (phosphate buffered saline) by a method known in the art or a method pursuant thereto. The resultant emulsion is then dispersed into an ophthalmic composition.

The mean diameter of particles in the emulsion is preferably from about 0.05 to about 0.3 μm.

[0022]

In the present invention, a commercially available fluorocarbons or fluorocarbon mixtures can be used. Examples of commercially available fluorocarbons include inert liquid FC-43, FC-75, FC-77, FC-78, FC-88 (Minnesota Mining Manufacturing Company), and examples of commercially available fluorocarbon emulsion include Fluosol® (Midori-Juji Co., Ltd.).

[0023]

A liquid fluorocarbon is also usable, which can be prepared according to a method described in, for example, JP

H02-271907, A or a method pursuant thereto.

[0024]

The sterilization of an oxygen-rich ophthalmic composition containing fluorocarbon is preferably carried out at any stage of the total process for preparing the formulation by a method not affecting the dispersing nature of fluorocarbon emulsion.

For example, the fluorocarbon emulsion or liquid fluorocarbon prepared as above is preferably sterilized before capturing oxygen. Fluorocarbons may condense upon heat sterilization, and, therefore, stabilization is conveniently conducted by a method known to prevent such a problem as shown in JP S48-26912, A.

[0025]

The step wherein fluorocarbon captures oxygen can be positioned at any stage of the total process for preparing the formulation. However, it can be preferably conducted under aseptic conditions after the sterilization.

The process for making fluorocarbons capture oxygen can be conducted by known methods, for example, a method comprising dissolving oxygen into fluorocarbon emulsion in an atmosphere of high oxygen partial pressure, a method comprising bubbling oxygen into fluorocarbon emulsion, or a method pursuant thereto.

[0026]

Because the specific gravity of fluorocarbons is between 1.5 and 2.5, an ophthalmic composition containing

fluorocarbon is preferably shaken before use.

[0027]

As an oxygen carrier, hemoglobin, modified hemoglobin, or a polymer thereof (hereinafter referred to as "hemoglobin etc.") can be used.

[0028]

Hemoglobin can be obtained, without limitation, by known methods, for example, a method described in JP H11-046759, A, or a method pursuant thereto from a raw material such as erythrocytes, concentrated erythrocyte composition derived from human beings or animals, especially from mammals specifically cow, pig, or the like.

[0029]

The oxygen carrier used in the present invention may be a polymer of Hemoglobin producible in accordance with a known method or a method pursuant thereto.

[0030]

Examples of modified hemoglobin includes those that can be obtained by binding hemoglobin to polyoxyethylene- or polyethylene- glycol, or the like; those that can be obtained by treating hemoglobin in the presence of a cross-linking agent such as glutaraldehyde or bis(3,5-bromosalicyl)fumarate as a cross-linking agent. Specific examples of modified hemoglobin include those wherein hemoglobin molecules are linked each other through alpha chains, or hem-binding albumin, and the like.

[0031]

The oxygen carrier used in the present invention may be a polymer of modified Hemoglobin producible in accordance with a known method or a method pursuant thereto.

[0032]

Hemoglobin etc. are dissolved in a solvent conventionally used in the art, such as physiological saline, PBS or Ringer solution containing lactic acid.

By elevating oxygen partial pressure, hemogulobin can be made to capture oxygen. The oxygen partial pressure should be adjusted depending on the intended amount of oxygen to be dissolved in an ophthalmic composition, but is preferably between about 40 mmHg and about 150 mmHg.

[0033]

It is preferred to sterilize an oxygen-rich ophthalmic composition of the present invention by a known method, such as sterile filtration and sterilization by heating. It is preferred to prepare the oxygen-rich ophthalmic composition under aseptic conditions after the sterilization.

[0034]

If hemoglobin undergoes oxidization to give methemoglobin (MetHb) during the process of producing oxygen-capturing hemoglobin or the storage before use, it would lose the capacity to bind to oxygen and results in the deficiency of oxygen transporting ability.

A known method may be used to avoid the above problem as much as possible.

In the present invention, a method can be applied

in which hemoglobin etc. are prepared and preserved at a low temperature (about 0°C-about 4°C). The reason thereof is that the oxidation rate of hemoglobin depends on temperature, and thus a low temperature can reduce the oxidation rate.

Further, the above problem can be avoided as much as possible by adjusting the pH value of the composition in the range between 6.5 and 9.0, because the oxidation rate of hemoglobin etc. depends on pH.

[0035]

Furthermore, the oxidation of hemoglobin etc. can be inhibited by adding a reducing agent harmless to human body such as ascorbic acid and the like.

A method is known, in which enzymatic reduction of hemoglobin etc. is conducted (*Biochimicaet Biophysica Acta* **310**:309-316, *Archives of Biochemistry and Biophysics* **77**:478-492). Said method may also be used in the present invention.

The addition of a specified alcohol can also reduce oxidization of hemogulobin etc. (Biotechnology and Applied Biochemistry 18:25-36). Said method may also be used in the present invention.

[0036]

Also, oxidation of hemoglobin etc. can be inhibited by attaching an appropriate protective ligand to enzyme binding site of hemoglobin etc. This can be used in the present invention.

Examples of protective ligand include gaseous

ligands such as nitric oxide (NO), non-gaseous competitive ligands such as "derivatives" of carbon monoxide, for example, nitroso compounds, isocyanide, and the like.

[0037]

As an oxygen carrier of the present invention, hemoglobin etc. can be encapsulated in a lipid endoplasmic reticulum.

[0038]

Lipid endoplasmic reticula are morphologically classified into three types, i.e., multilayered liposomes consisting of multiple lipid membranes (Multilamellar Liposome or Vesicle (MLV)), small liposomes consisting of monolayer (Single Compartment Liposome or Small Unilamellar Vesicle (SUV)) and large liposomes consisting of monolayer (Large Unilamellar Vesicle (LUV)). For the present invention, small liposomes (SUVs) are preferred.

As a lipid composing an endoplasmic reticulum, phosphatidyl choline (PC), phosphatidyl serine (PS), phosphatidyl ethanolamine (PE), phosphatidyl inositol (PI), lyzophosphatidyl choline (LPC), ganglioside (G), cardiolipin (CL), sphingomyelin (SM), dipalmitoylphosphatidyl choline (DPPC), distearoylphosphatidyl choline (DSPC), phospatidic acid (PA) or phosphatidyl glycerol (PG), or those derived from lipids above through the hydrogenation according to a conventional method are included.

[0039]

In addition to a lipid, one or more known substances

used in the art may optionally be added.

For example, cholesterol (CHOL), dicetyl phosphate (DCP), stearylamine (SA), polyethyleneglycol (PEG), and the like may be added.

Cholesterol is also useful in the formation of endoplasmic reticulum, the stabilization of membrane, and the adjustment of permeability of liposome membrane.

Further, since dicetylphosphate and stearylamine are charged positively and negatively, respectively, they are useful as a charge donor and thereby adjusting the contact of liposomes and cells.

Polyethyleneglycol is also useful in the prevention of agglutination among liposomes.

However, all of these substances are not necessarily essential for the formation of endoplasmic reticulum.

[0040]

Furthermore, tocopherol analogs can be added during the formation of endoplasmic reticula.

Tocopherol (vitamin E) is a biological element having nonspecific antioxidative effect and can contribute to the stabilization of endoplasmic reticulum membrane or hemoglobin molecules as an antioxidant.

In particular, it can be a significant component for the endoplasmic reticulum containing unsaturated lipids.

[0041]

It is also preferable to add into a lipid

endoplasmic reticulum an inhibitor for preventing the conversion of hemoglobin into MetHb.

Examples of such inhibitors include sugars involved in the anaerobic glycolytic pathway, a precursor capable of preventing the generation of MetHb.

A specific example of a sugar involved in the anaerobic glycolytic pathway is malic acid. Examples of precursors capable of preventing the generation of MetHb include active enzyme scavengers (removers) such as cytochrome b5, NADH-cytochrome b5 reductase, catalase, superoxide dismutase, glutathione peroxidase, or the like.

[0042]

Also, an allosteric factor of hemoglobin can be included in a lipid endoplasmic reticulum together with hemoglobin etc.

When hemoglobin etc. are encapsulated in lipid endoplasmic reticula, the former can be present in the aqueous phase of the latter at high concentration, which advantageously results in the reduction of the viscosity as well as colloid osmotic pressure of the resultant ophthalmic composition.

However, a lipid endoplasmic reticulum containing purified hemoglobin etc. has much higher affinity to oxygen than erythrocyte and shows extremely high binding activity with oxygen, and hence hardly releases oxygen under low oxygen partial pressure (Circulatory Shock, 31,431 (1990)).

To improve the oxygen transporting capacity of a lipid endoplasmic reticulum containing hemoglobin etc., in

particular to reduce the affinity of a lipid endoplasmic reticulum containing hemoglobin etc. to oxygen under low oxygen partial pressure, an allosteric factor of hemoglobin is preferably included in a lipid endoplasmic reticulum together with hemoglobin etc.

[0043]

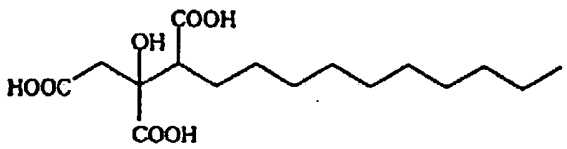
Examples of allosteric factor of hemoglobin include 2,3-diphosphoglyceric acid, inosithexaphosphoric acid (IHP), polycarboxylic acid, or condensed phosphoric acid.

Further, an electrolyte comprising chloride ion as the negative ion and an organic phosphoric acid derivative are also usable. Examples of electrolyte comprising chloride ion as the negative ion include sodium chloride, potassium chloride, calcium chloride and magnesium chloride. Examples of phosphoric acid derivative include adenosine triphosphate (ATP), adenosine diphosphate (ADP) and pyridoxal-5-phosphoric acid (PLP).

[0044]

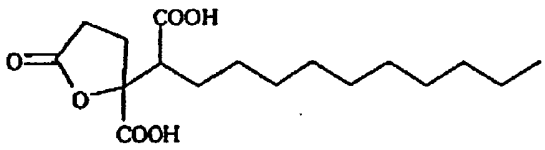
As an allosteric factor, a compound of the formula (2):

[Chemical formula 2]




or a compound of formula (3):

[Chemical formula 3]




may be used.

Since both the compounds (2) and (3) have two asymmetric carbon atoms, plural stereoisomers (diastereomer and optic isomers) exist. For the composition of the present invention, there can be used not only a purified isomer but also a mixed compounds comprising any isomers at any proportion, racemate or diastereomer.

Compounds of the formulae (2) and (3) can be used alone or in combination at optional ratio.

Examples of physiologically acceptable base addition salt of the compounds of the formulae (2) and (3) include salts with metal such as sodium salt, potassium salt or calcium salt; salts with organic base such as ammonium salt; methyl amine salt, ethyl amine salt or trimethyl amine salt.

[0045]

The compounds of the formulae (2) and (3) are known in the art and can be obtained from commercially available ones or produced by a known method or a method pursuant thereto.

[0046]

The process of preparing a lipid endoplasmic reticulum is known in the art and is not limited to any methods. Lipid endoplasmic reticulum can be prepared in a conventional manner though, there are certain methods preferred, for example, glass beads agitation method, cholic acid elimination method,

reversed phase evaporation method, French press method, $Ca^{2+}$ fusion method, pearl pumping method, or dehydrate-rehydration method, considering that hemoglobin etc. to be encapsulated are liable to undergo oxidation and to change to methemoglobin which lacks oxygen transporting capacity under the influence of temperature, light, hydrogen ion concentration, dissolved gas or metal ions.

[0047]

The resultant endoplasmic reticula is then dissolved in a solvent conventionally used in the art, such as physiological saline, PBS or lactic acid containing Ringer solution.

By increasing the oxygen partial pressure of the solvent, hemoglobin or modified hemoglobin can be made to capture oxygen. The oxygen partial pressure should be adjusted appropriately depending on the amount of oxygen desired to be dissolved in an ophthalmic composition though, it is generally preferred to be ranging between about 40 mmHg and about 150 mmHg.

[0048]

It is preferred to subject the oxygen-rich ophthalmic composition of the present invention to sterilization using a know sterilizing method such as sterile filtration and sterilization by heating at any stage of the total process for preparing the formulation. It is preferred to prepare the oxygen-rich ophthalmic composition under aseptic conditions after the sterilization.

[0049]

When using, as oxygen carriers, endoplasmic reticula including hemoglobin etc. in the present invention, they are preferably stored in a frozen state.

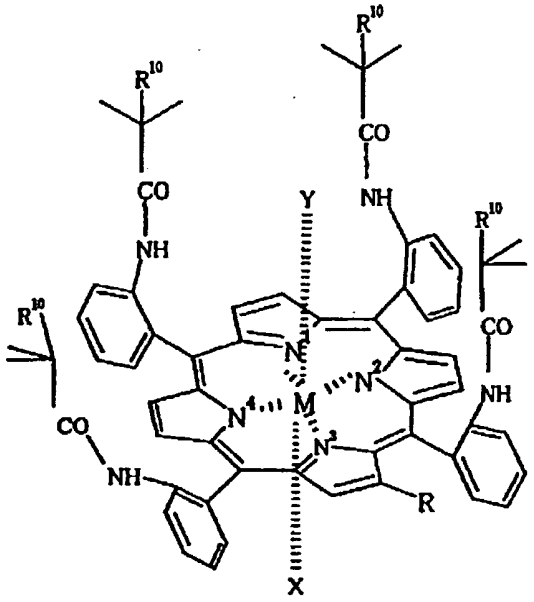
To keep them stable for a long term, it is preferred to add a freezing protecting agent such as sugar or glycerol.

Further, freezing and preservation of endoplasmic reticula can be carried out after adding a compound showing inhibitory effect on agglutination and fusion of endoplasmic reticulum as an stabilizer, which compound is selected from oligo glycolipids, wherein a long chain alkyl is bound to an oligosaccharide through ester binding, and glycolipids, wherein a long chain alkyl is bound to terminal anomer site of an oligosaccharide chain through ether, amide or ester binding.

[0050]

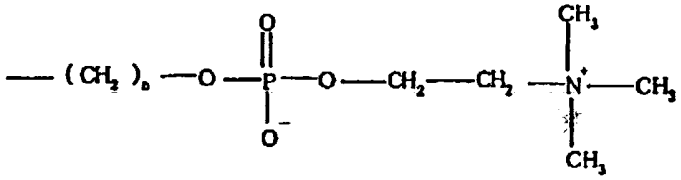
Porphyrin complexes to be used as carriers in the present invention are derived from ferrous porphyrin (iron (II) porphyrin) complex present in hemoglobin or myoglobin through modification for improving the stability at room temperature, and are shown by the formula (4):

[Chemical formula 4]



wherein R is hydrogen or a substituent, M is a transition metal ion of fourth or fifth period. The formula (4) above also shows a complex wherein a transition metal ion is absent and a hydrogen atom is bound to each nitrogen atom represented by $N^2$ and $N^4$. $R^{10}$ is a substituent selected from the group consisting of $C_{1-18}$ alkyl and a group of the formula (5):

[Chemical formula 5]



wherein n is the number of methylene group and is selected from the integer of 1-20. X and Y represent a ligand of transition metal ion.

[0051]

A transition metal ion M of fourth or fifth period located in the center of the porphyrin ring of a porphyrin complex represented by the formula (4) is preferably divalent or trivalent iron ion, or divalent cobalt ion.

[0052]

Among porphyrin complexes of the formula (4), a complex wherein M is iron(II) ion, X and Y each are hydrogen, and $R^{10}$ is methyl, i.e., 5,10,15,20-tetrakis(α,α,α,α-o-pivalamidephenyl)porphyrin iron(II) complex, can bind reversibly to an oxygen molecule(s) at room temperature in the presence of an axis base such as 1-alkyl imidazole or 1-alkyl-2-methyl imidazole in a solvent such as benzene, toluene or an organic solvent such as N,N-dimethylformamide. However, oxygen-absorption-desorption capacity is exhibited only in an organic solvent, and thus this complex is difficult to be applied to an ophthalmic composition of the present invention.

Thus, this complex is preferably included into a lipid endoplasmic reticulum as described above. By doing so, a porphyrin complex of this class can exert the similar capacity as above even under a physiological conditions (aqueous phase, about pH7.4, about 40 ℃ or below).

[0053]

A porphyrin complex wherein $R^{10}$ is a substituent shown by the formula (5), which has a structure similar to phospholipid, forms an endoplasmic reticule of bimolecular film when dispersed into water together with phospholipid. Then,

said complexes are dispersed evenly on the side of a hydrophobic layer.

Since there is no drawback of showing oxygen absorption-desorption capacity only in an organic solvent different from a porphyrin complex wherein $R^{10}$ is a methyl group, this complex can be preferably used as an oxygen carrier of the present invention.

Such a porphyrin complex having a structure similar to phospholipid can further be included in a lipid endoplasmic reticulum. When encapsulated in a lipid endoplasmic reticulum, porphyrin complexes can perform the absorption-desorption function repeatedly and stably for a long period of time.

[0054]

Examples of the substituent R at the 2-position of porphyrin ring of a porphyrin complex of the formula (4) include -R'-CHO, -R'-COOH, -R'-COO-R", -COO-R", -R'-$NH_2$, -R'-$SO_3H$, $C_{6-20}$ aryl group, hydroxyl group, halogen, formyl group, carbonyl group, amino group, sulfonyl group, azide group, oxy carbonyl chloride or imidazole derivatives.

[0055]

In the above formula, R' and R" represent an aliphatic hydrocarbon chain group. Preferably, the number of carbon atoms in R' is 1-10 while that of R" is 1-20. R and R" may be the same or different from each other.

The aliphatic hydrocarbon chain group may be straight or branched, and saturated or unsaturated.

Specifically, examples of such group include an

alkyl group such as methyl group, ethyl group, n-propyl group, isopropyl group, n-butyl group, isobutyl group, tert-butyl group, pentyl group, isopentyl group, tert-pentyl group, hexyl group, 1,1-dimethylpropyl group, 3-methyl-3-butenyl group, and the like; an alkenyl group such as vinyl group, allyl group, 1-propenyl group, isopropenyl group, 2-butenyl group, 1,3-butadienyl group, 2-pentenyl group, and the like; an alkynyl group such as ethynyl group, 2-propynyl group, 1-butynyl group, 2-butynyl group, or the like.

Further, a substituent may have both the double- and triple-bonds as shown by 2-pentene-4-nylyl group.

The R″ is preferably a straight chain aliphatic hydrocarbon group of carbon number of 1-18. The said chain aliphatic hydrocarbon group may be substituted with halogen. The position and the number of substituents can be selected freely without limitation as far as it is chemically acceptable.

[0056]

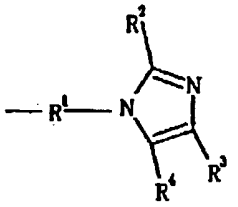
The term "$C_{6-20}$ aryl group" refers to an aromatic hydrocarbon group which may be partially saturated.

Examples include phenyl group, benzyl group, biphenyl group, indenyl group, naphthyl group, or a partially saturated derivatives thereof such as 2,3-dihydroindenyl group or 1,2,3,4-tetrahydronaphthyl group, and the like.

[0057]

Imidazole derivatives as substituents are shown by the formula (6):

[Chemical formula 6]



wherein $R^1$ is $C_{1-20}$ alkylene which is optionally be intervened by -OCONH-, -CONH- or -NHCO- and $R^2$, $R^3$ and $R^4$ are independently hydrogen or $C_{1-6}$ alkyl group.

[0058]

Although an imidazole derivative is widely used as an axis base, the addition of the derivative in an excess amount may result in the expression of its own pharmacological effect or toxicity. Further, in the case where an imidazole derivative is included in a lipid endoplasmic reticulum, it can be one of factors that make the configuration of lipid endoplasmic reticulum unstable.

Accordingly, introducing an imidazole derivative into a molecule as a substituent through covalent bond is advantageous in making the amount of axis base as little as possible.

[0059]

The introduction of an appropriate substituent at the 2-position of porphyrin ring can form a covalent bond between a porphyrin complex and a highly fat soluble substance.

For example, a porphyrin complex bound to a fatty acid through an ester or amide bond can show an improved

fat-solubility and hence high solubility in hydrophobic region of a lipid endoplasmic reticulum, which means that such a porphyrin complex can be included into a lipid endoplasmic reticulum easily.

[0060]

Furthermore, the introduction of the appropriate substituent at the 2-position of porphyrin ring makes it possible to prepare a porphyrin complex having both lipophilic and hydrophilic properties.

Thereby, when the complex is encapsulated in an endoplasmic reticulum, for example, agglutination or deposition of the polyhyrin complex after the collapse of the endoplasmic reticulum, which takes places when using said complex as an ophthalmic composition of the present invention, can be prevented.

[0061]

In the case of a porphyrin complex of the formula (4) wherein a transition metal ion M of fourth or fifth period located in the center of the porphyrin ring of a porphyrin complex represented by the formula (4) is a divalent metal, two hydrogen atoms generally form coordinate bonds. Both or either of their ligands may be an imidazole derivative(s).

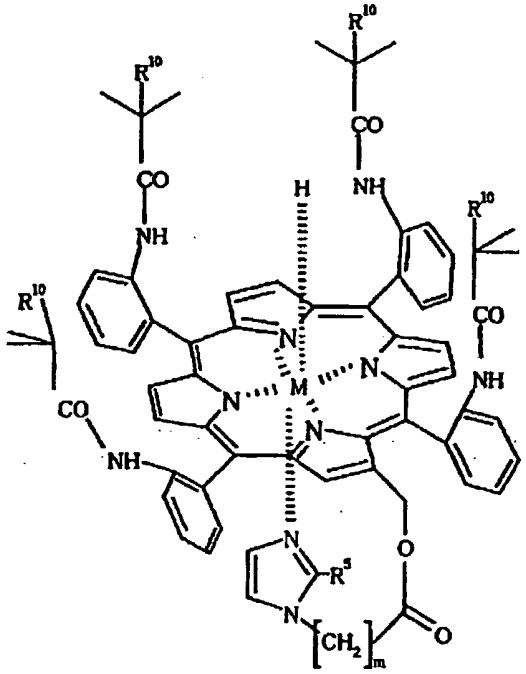
Thereby, it is possible to reduce the amount of axis base to be added even to the limiting value.

[0062]

Examples of porphyrin complexes used in the present invention include those wherein an imidazole derivative, as a

ligand, is attached to the transition metal M at the center of porphyrin ring through an ester bond (1mole : 1 mole), as shown by the formula (7):

[Chemical formula 7]

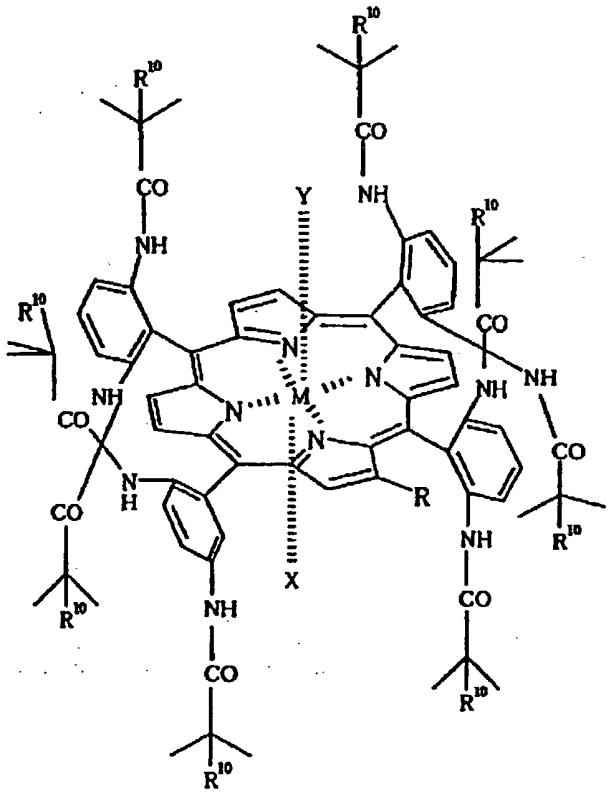



wherein m is an integer of 7-17 and $R^5$ is hydrogen or methyl group, and M and $R^{10}$ are as defined above. One of advantages of the compound above is that the amount of axis base to be added can be reduced to the limiting value.

[0063]

A compound having bulky substituents introduced at both sides of porphyrin ring, which is shown by the formula (8):

[Chemical formula 8]



wherein M, X, Y and $R^{10}$ are as defined above is also useful.

In this case, a porphyrin complex having a bulky substituent at only one side of porphyrin ring is not suited as a carrier for the present invention because such a complex is liable to form μ-dioxo dimmer to be oxidized to iron(III) porphyrin complex lacking the oxygen binding activity. By introducing bulky substituents at both sides of porphyrin ring, oxidation and deterioration of the complex can be prevented, thus making it possible to provide a more stable oxygen carrier.

[0064]

Further, a porphyrin complex of the formula (7) or (8) can be included in a lipid endoplasmic reticulum as mentioned above.

[0065]

The porphyrin complexes used as an oxygen carrier of the present invention can be produced according to a method such as that described in JP S59-162924, A.

[0066]

In addition to the porphyrin complex shown by the formula (4), (7) or (8) above, the present invention can use as an oxygen carrier known synthetic iron(II) porphyrin complexes having oxygen absorption-desorption capacity similar to iron(II) porphyrin complexes present in hemoglobin or myoglobin, as described in J. P. Collman, *Accounts of Chemical Research*, 1977, **10**, 265; Basolo, B. M. Hoffman, J. A. Ibers, *ibid* 1975, **8**, 384.

[0067]

The oxygen carrier of the present invention can be a polymer of the above-mentioned porphyrin complex. Said polymer can be produced through the polymerization of porphyrin complexes in accordance with a known method or a method pursuant thereto.

[0068]

In the porphyrin complexes represented by the formula (4), a complex wherein M is iron(II) ion, X and Y each are hydrogen, and $R^{10}$ is methyl, i.e., 5,10,15,20-tetrakis(α,α,α,α-o-pivalamidephenyl)porphyrin iron(II)

complex exhibits oxygen-absorption-desorption capacity only in an organic solvent and is thus difficult to be applied to an ophthalmic composition of the present invention.

However, an oxygen carrier wherein the complex is encapsulated in a lipid endoplasmic reticulum is stably present in an aqueous system. Further, a porphyrin complex having a structure similar to phospholipid forms an endoplasmic reticule of bimolecular film with high compatibility when dispersed into water together with phospholipid. Since said complexes are dispersed evenly on the side of a hydrophobic layer, such porphyrin complexes are stably present in the aqueous system.

Accordingly, such porphyrin complexes are dissolved in a solvent conventionally used in the art, such as physiological saline, and are then made to capture oxygen by a known method, such as bubbling oxygen into the complexes-dissolved solvent under high pressure, or by a method pursuant thereto.

[0069]

The emulsion or solution containing the oxygen carrier may be used as such as an opthalmic composition of the present invention, and it may further be sterilized if desired. The emulsion or solution may also be diluted with distilled water or physiological saline. Alternatively, the following ingredients may further be added to the emulsion or solution.

[0070]

The thus prepared ophthalmic composition enriched with oxygen of the present invention is preferably sterilized

at any stage of the total process for preparing the formulation by a known method such as sterile filtration or sterilization by heating. The steps following the sterilization are preferably conducted under an aseptic condition.

[0071]

The oxygen-rich ophthalmic composition of the present invention can be produced by a method for increasing the dissolved oxygen concentration without using any oxygen donors.

Examples include a method wherein oxygen is incorporated into an ophthalmic composition under oxygen gas atmosphere. As a method of incorporating oxygen, a method wherein oxygen is bubbled into a composition; and a method wherein oxygen is allowed to dissolve into a composition under high oxygen partial pressure are included.

Further, a method wherein oxygen is bubbled into a composition under high oxygen partial pressure is included.

In this context, "high pressure" refers to about 1.2 to 5 atmospheric pressure.

[0072]

Further, a method in which an ophthalmic composition is prepared at a low temperature is also included. The reason thereof is that dissolved oxygen in water increases in quantity as the temperature goes down.

The temperature is preferably between about 0 ℃ - 5 ℃ in the preparation of an ophthalmic composition enriched with oxygen of the present invention.

The above-mentioned processes can be conducted in combination. That is, a method wherein oxygen is bubbled into an ophthalmic composition directly under a condition of low temperature and oxygen atmosphere, or a method wherein oxygen is allowed to dissolve into the composition under a condition of low temperature and high oxygen pressure can be used.

[0073]

Besides oxygen or oxygen donor inorganic salts, sugars, vitamins, amino acids, epidermal growth factors and oxygen scavengers, tissue activating agents or metabolic enhancers, agents for treating disorder of corneal epithelium layer or enhancing expansion of corneal epithelium layer, coenzymes or cell differentiation promoter may be contained in a conventionally applied ratio.

Especially preferable ingredients include glucose, hyaluronic acid, nicotinamide-adenine dinucleotide (NAD), flavin adenine dinucleotide (FAD), vitamin Ds, aspartic acid and salts thereof, taurine, SOD or SOD-like activity substrates, and the like.

[0074]

Inorganic salts include, for example, potassium chloride, calcium chloride, sodium chloride, sodium bicarbonate, sodium carbonate, magnesium sulfate, disodium hydrogenphosphate, sodium dihydrogenphosphate, potassium dihydrogenphosphate, and the like.

Sugars include, for example, monosaccharides such as glucose etc., disaccharides such as sucrose etc., or

polysaccharides such as dextran, cyclodextrin, hyaluronic acid etc., and the like.

Vitamins include, for example, nicotinic acid, nicotinamide-adenine dinucleotide (NAD), flavin adenine dinucleotide (FAD), vitamin Ds (preferably, vitamin $D_3$), vitamin $B_2$, vitamin $B_6$, vitamin $B_{12}$, panthenol, calcium pantothenate, sodium pantothenate, vitamin A acetate, vitamin E acetate, retinol palmitate, and the like.

Amino acids include, for example, aspartic acid and salts thereof such as magnesium potassium L-aspartate, potassium L-aspartate or magnesium L-aspartate, sodium chondroitin sulfate, and the like.

[0075]

Epidermal growth factors include, for example, taurine (aminoethylsulfonic acid).

Active oxygen scavengers include, for example, substances having SOD or SOD-like activity.

Tissue activating agents or metabolite activating agents include aspartic acid and salts thereof.

Agents for treating disorder of corneal epithelium layer or enhancing expansion of corneal epithelium layer include polysaccharides such as hyaluronic acid, and biological energy sources such as glucose, and the like.

Coenzymes include, for example, vitamins such as NAD or FAD.

Cell-differentiation accelerating agents include, for example, vitamin Ds (preferably vitamin $D_3$), and the like.

[0076]

Additionally, the present oxygen-rich ophthalmic compositions may contain components ordinary used for ophthalmic compositions in pharmaceutical acceptable amounts. For example, a component(s) selected from the followings may be included, as need arises:

(a) redness relievers such as epinephrine, epinephrine hydrochloride, ephedrine hydrochloride, tetrahydrozoline hydrochloride, naphazoline hydrochloride, naphazoline sulfate, phenylephrine hydrochloride, methylephedrine hydrochloride, and the like;

(b) neostigmine methylsulfate;

(c) anti-inflammatory agents such as glycyrrhizinate dipotassium, epsilon-aminocapronic acid, allantoin, berberine chloride, berberine sulfate, sodium azulenesulfate, zinc sulfate, zinc lactate, lysozyme chloride, and the like;

(d) anti-histamines such as diphenhydramine hydrochloride or chlorpheniramine maleate, and the like;

(e) preservative, for example, quaternary ammonium salt-type preservatives such as benzalkonium chloride or benzethonium chloride; guanidine-type preservatives such as chlorhexidine hydrochloride, chlorhexidine gluconate, dodecylguanidine chloride or 6-acethoxy-2,4-dimethylmetadioxane, chlorobutanol, and the like;

(f) thickeners such as polyvinyl alcohol, polyvinylpyrrolidone, hydroxyethyl cellulose, hydroxypropylmethyl cellulose, hydroxymethyl cellulose, and

the like;

(g) buffering agents such as boric acid, and the like;

(h) pH adjusting agents such as borate, and the like;

(i) solubilizing agents such as polyoxyethylene hardened castor oil, and the like;

(j) ethylenediaminetetraacetatic acid (EDTA) or salts thereof; and

(k) refreshing agents such as camphor, menthol, and the like.

[0077]

It is preferred to adjust the pH of the composition of the present invention at about 4 to 9.

[0078]

When using an oxygen-permeable container, there is a drawback in that the dissolved oxygen concentration in the oxygen-rich ophthalmic composition decreases with time. Thus, in the present invention, use of a container impermeable or less permeable to oxygen is conceived so as to solve this problem.

A container impermeable to oxygen means a container that lacks oxygen-permeability, while a container less permeable to oxygen means a container with low oxygen-permeability.

Such container includes those made from a high gas barrier material, those composed from a high gas barrier material, or those comprising a high gas barrier material.

Although it is preferred to use only a high gas barrier material for containers impermeable or less permeable

to oxygen, in view of cost, containers wherein polyethylene and a high gas barrier material are multilayered may be applied, provided that such containers do not influence the effect of the present invention.

[0079]

Further, a container impermeable or less permeable to oxygen may be encased in a package impermeable or less permeable to oxygen.

A package impermeable to oxygen means a package that lacks oxygen permeability, while a package less permeable to oxygen means a package with low permeability. For example, packages made from or composed of aluminum foil or a high barrier material or packages comprising a high gas barrier material are included. The package may be single or multilayered film. Further, the package can be prepared by coating a film made from a material of polyvinyl alcohols or polyamides basis with aluminum, metal oxide such as aluminum oxide, or ceramics such as silicon oxide by vapor deposition method, or with polyvinylidene chloride, or the like.

Although it is preferred to use only a high gas barrier material for containers impermeable or less permeable to oxygen or packages less permeable to oxygen, in view of cost, containers wherein polyethylene and a high gas barrier material are multilayered may be applied, provided that such containers or packages do not influence the effect of the present invention.

[0080]

Further, after the ophthalmic composition of the present invention is charged in an oxygen-permeable container, the container may be encased in an oxygen-impermeable or -less permeable container.

The oxygen-permeable container means a container made from an oxygen-permeable material and free from high gas barrier material.

Examples of the oxygen-permeable material include Teflon, polystyrene, polyethylene, polypropylene and the like. It is preferred to replace the void space formed between the container and the package with oxygen.

[0081]

Preferred high gas barrier material is a material lacking oxygen permeability or having low oxygen permeability. Specifically, oxygen-permeability of such a material is preferably 0-100cc·20μm/$m^2$·24hr·atm (measured at 20°C, 65%RH) is preferable, and the one with the oxygen permeability of 0-10cc·20μm/$m^2$·24hr·atm is more preferable.

Thermoplastics are especially suitable as high gas barrier materials and specific examples include polyamide (nylon 610 etc.), EVOH (ethylene-vinyl alcohol copolymer), PVDC (polyvinylidene chloride), PEN (polyethylene naphthalate), PET (polyethylene terephthalate), and the like. EVOH is preferred above all. These materials having elevated gas barrier nature can be used after drawing.

[0082]

The oxygen-impermeable or -less permeable

container, or oxygen-impermeable or -less permeable package containing EVOH, a preferred high gas barrier material in the present invention, includes those made from only EVOH, those made from plastic such as polyethylene and a high gas barrier material, which are multilayered, those made from resin into which EVOH has been kneaded.

[0083]

Further, it is preferred to replace the room between the package and the container with oxygen gas. The amount of oxygen to be filled in the room between an oxygen-permeable container and a package impermeable or less permeable to oxygen should be sufficient to maintain the desired equilibrium between the oxygen atmosphere in the void space and the dissolved oxygen in the ophthalmic composition in an airtight environment. It is preferred to replace about 50% or more of the space volume with oxygen gas.

Because the equilibrium between the oxygen atmosphere in the room between the oxygen-permeable container and the package and the dissolved oxygen in the ophthalmic composition is thus achieved, the dissolved oxygen can be maintained for a long term.

[0084]

A composition of the present invention, when a container is encased in a package, can be prepared according to a process comprising, for example, the following steps:
(1) filling an ophthalmic composition containing an oxygen carrier or an ordinary ophthalmic composition into a container;

(2) incorporating oxygen into the composition above;

(3) closing tightly the container above and encasing the container in a package impermeable or less permeable to oxygen;

(4) filling oxygen into the room between the container and the package and sealing.

Alternatively, it can be prepared according to a process comprising the following steps:

(1) incorporating oxygen into an ordinary ophthalmic composition;

(2) filling the oxygen-rich ophthalmic composition prepared in (1) above into a container;

(3) closing tightly the container above and encasing the container in a package impermeable or less permeable to oxygen; and

(4) filling oxygen into the room between the container and the package and sealing.

The sterilization step can be positioned before or after of any one of steps (1) to (4) of processes above; however, preferred position is before the step (2) in case of the former process, and before the step (1) in case of the latter process. The steps following the sterilization are preferably conducted under an aseptic condition.

[0085]

In the present invention, the oxygen-rich ophthalmic composition is particularly preferably filled in an oxygen-impermeable or -less permeable container.

However, it is also possible to prepare an

oxygen-rich ophthalmic composition using an oxygen-permeable container. That is, a conventional ophthalmic composition is filled in an oxygen-permeable container; the container is encased in an oxygen-impermeable or -less permeable package; and the void space between the container and the package is replaced with oxygen. Thereby, oxygen is gradually dissolved in the ophthalmic composition and an oxygen-rich ophthalmic composition is prepared.

[0086]

There is no restriction on the form, size, and the like of container into which the present composition is filled; however, such containers generally have the form of rectangle or cylinder with a capacity ranging from about 0.2mL to 30mL, which are conveniently applied to the present invention.

[0087]

There is no specific restriction on the form, size, and the like of package provided that it can accommodate the container above.

In case that oxygen is filled in the room between a package and a container, the package preferably has a shape and size suited to provide a room of sufficient volume between the container and the package after the container is encased. In general, a package is preferably has about 1.2 to about 2 times the volume of the above-mentioned plastic container.

[0088]

It is preferred that a composition of unit dosage form is included in a container that is encased in a package.

[0089]

In the process for producing an oxygen-rich composition of the present invention, it is preferred that the steps before the step for filling the composition into a container and also the step for filling the composition into a package are conducted under low temperature or an oxygen atmosphere.

In case that the sterilization is done beforehand, the filling step is preferably conducted under aseptic conditions.

[0090]

The ophthalmic composition of the present invention is used as, for example, ophthalmic solutions, eye wash or contact lens-fitting solution, or the like.

Eye wash is used for washing eyes after wearing contact lenses or swimming, in order to prevent eye disease or remove annoyance of eyes, or to remove pollen or dust. In particular, it is preferred to use eye wash before or after using contact lenses.

Contact re-wetting and/or wetting solution is used for facilitating wearing of contact lenses. It is usually used before wearing contact lenses in order to wet both surfaces of contact lenses or inner surfaces thereof.

The oxygen-rich ophthalmic composition of the present invention can be used for dry eye, supplement of tear, deficiency of tear or the like. Those who wear contact lenses can preferably use this composition.

[0091]

The oxygen-rich composition of the present invention can be used for disorders caused by oxygen deficiency while wearing contact lenses and so on. Said disorders include punctate disorders such as keratitis superficialis punctate; corneal vascularization; plane disorder including disorders of corneal epithelium such as epithelial erosion or acute corneal epithelial edemas; and also other disorders such as vascular infiltration or disorders of corneal endothelium.

[0092]

Example 1: Eye drop

| | |
|---|---|
| Sodium chloride | 0.700g |
| Potassium chloride | 0.100g |
| Boric acid | 1.000g |
| Borate | 0.200g |
| Disodium Edetate | 0.050g |
| Sorbic acid | 0.1g |
| Solution of 0.1 N sodium hydroxide in water | p.r.n. |
| 0.1 N Hydrochloric acid | p.r.n. |
| Sterile purified water | p.r.n. |
| Total | 100ml |

Disodium edetate was dissolved gradually in 80 ml of water. To the disodium edetate solution were added boric acid and borate. After sodium chloride and potassium chloride were dissolved, sorbic acid was added and dissolved in the solution. The resultant solution was adjusted to about pH 7.4 using 0.1 N sodium hydroxide and 0.1 N hydrochloric acid solutions, and the total volume was adjusted to 100 ml.

Oxygen gas was bubbled in the solution under oxygen

gas atmosphere at 1 atm, 20°C to supersaturate the dissolved oxygen. Then, under oxygen gas atmosphere, the solution was filtered through cellulose acetate filter (0.2 μm pore-size), filled into sterile plastic containers of polyethylene naphthalate and the void space was replaced with oxygen, the container was encased in a package of a composite film mainly consisting of polyvinyliden chloride, and the room between the container and the package was replaced with oxygen. The eye drop of the present invention was obtained after heat-seal. The dissolved oxygen concentration at 20°C was 40 mg/L, which was about 4.5-fold of saturated dissolved oxygen concentration.

[0093]

Example 2:

A solution was prepared according to the same method as described in Example 1. Oxygen gas was bubbled in the solution under oxygen gas atmosphere at 1 atm, 20°C to supersaturate the dissolved oxygen. Under air atmosphere, the solution was then filtered through cellulose acetate filter (0.2 μm pore-size) and filled into sterile plastic containers of polyethylene naphthalate, the eye drop of the present invention was obtained after heat-seal. The dissolved oxygen concentration at 20°C was 9 mg/L.

[0094]

Example 3:

A solution was prepared according to the same method as described in Example 1. Oxygen gas was bubbled in the solution under oxygen gas atmosphere at 1 atm, 5°C to

supersaturate the dissolved oxygen. The solution was filtered through cellulose acetate filter (0.2 μm pore-size) and filled into sterile plastic containers of polyethylene terephthalate and the container was heat-sealed to provide the eye drop of the present invention. The dissolved oxygen concentration at 5°C was 10 mg/L.

[0095]

Example 4:

A solution was prepared according to the same method as described in Example 1. Oxygen gas was bubbled in the solution under oxygen gas atmosphere at 5 atm, 5°C to supersaturate the dissolved oxygen. Under oxygen gas atmosphere at 5°C, the solution was filtered through cellulose acetate filter (0.2 μm pore-size) and filled into sterile plastic containers (EVOH) and then worked up as described in Example 1 to provide the eye drop of the present invention. The dissolved oxygen concentration at 20°C was 180 mg/L, which was about 20-fold of saturated dissolved oxygen concentration.

[0096]

Example 3:

To 60 ml of distilled water were added 2.5 g of glycerin and 1.5 g of polyoxyethylene hardened castor oil (Nikko chemicals Inc.), and the mixture was heated at 65°C to dissolve the components. To the solution was added gradually 20 g of fluorocarbon (Inert Liquid FC-75, Minnesota Mining & Manufacturing Co.) pre-warmed at 60 °C by injection to provide a crude emulsion.

Separately, 0.05 g of sodium edetate was dissolved gradually in 20 ml of water. To the sodium edetate solution were dissolved 1.0 g of boric acid and 0.2 g of borate, and 0.7 g of sodium chloride and 0.1 g of potassium chloride were dissolved. After 0.1 g of sorbic acid was added and dissolved, the pH of the solution was adjusted to about 7.4 with 0.1 N sodium hydroxide and 0.1 N hydrochloric acid.

This solution was added to the above crude emulsion and pH was adjusted to about 7.4 with 0.1 N sodium hydroxide and 0.1 N hydrochloric acid. The total volume was then adjusted to 100 ml by adding distilled water.

This solution was circulated with a high pressure emulsifier (Manton-Gaulin Emulsifier) under pressure at 450 atm for 5 minutes to provide an emulsion.

Oxygen gas was bubbled in the solution at 1 atm, 20°C for 30 minutes to make the oxygen-carrier, Inert Liquid FC-75 (Minnesota Mining & Manufacturing Co.) capture oxygen. The amount of captured oxygen was 15 mg/L.

Under oxygen gas atmosphere, the solution was then filtered through cellulose acetate filter (0.2 μm pore-size) and filled into a sterile plastic container of polyethylene naphthalate, which container was encased in a package of composite film mainly consisting of polyvinyliden chloride and the room between the container and the package was replaced with oxygen. The eye drop of the present invention was obtained after heat-seal.

[0097]

Test Example 1: Corneal Edema Inhibition Test

A contact lens of PMMA (polymethylmethacrylate) adapted to a rabbit cornea was applied.

The eye drops prepared in Examples 1-3 were instilled into eye 10 times every 30 minutes and 5 hours later, the corneas were removed and excised to prepare sections. The sections were stained with HE staining (hematoxylin eosin staining) and observed under optical microscope.

As control, saline was instilled into eye 10 times every 30 minutes for comparison. The corneas were stained with HE staining as described above and observed under optical microscope.

[0098]

As shown in Fig. 1, when saline was instilled, edema was observed in parenchyma portion of cornea. On the other hand, as shown in Fig. 2, when the eye drop prepared in Example 1 was instilled, little edema was observed. In the cases where the eye drop of Example 2 or 3 is used, little edema was also observed.

These results show that the ophthalmic compositions of the present invention are effective on the oxygen deficiency of cornea and can prevent, alleviate or treat the corneal disorders caused by the oxygen deficiency.

[0099]

Effect of the invention:

Disorder or disease in ophthalmologic field attributable to oxygen deficiency in cornea can effectively

prevented, alleviated or treated by an ophthalmic composition enriched with oxygen.

Thereby, it is possible to provide an ophthalmic composition effective on oxygen deficiency of cornea while wearing contact lenses, especially soft contact lenses, or on oxygen deficiency of cornea represented by dry eye.

Brief explanation of the drawings:

Fig. 1 is a copy of micrograph of cornea of a rabbit treated with physiological saline.

Fig. 2 is a copy of micrograph of cornea of a rabbit treated with eye drop of the present invention prepared in Example 1.

Document name: Drawings

Fig. 1

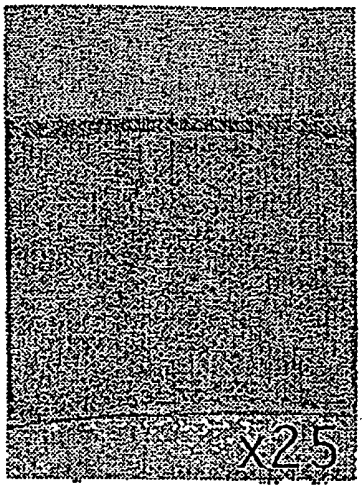
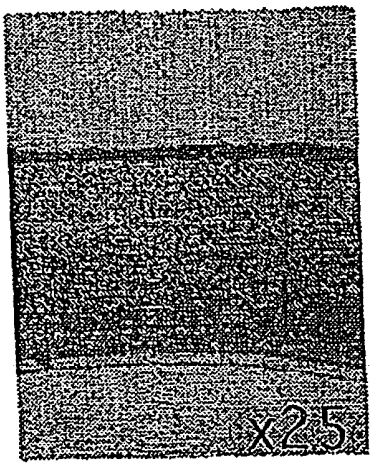



Fig. 2



Document name: Abstract

Summary:

Object: To provide an oxygen-rich ophthalmic composition for effectively preventing, alleviating, or treating disorder or disease in ophthalmologic field attributable to oxygen deficiency in cornea.

Solution: An ophthalmic composition characterized in that it comprises oxygen and/or an oxygen donor, and the amount of oxygen present in the composition is 10 mg or above per liter of the ophthalmic composition, or the content of oxygen is at least 95% of the saturated dissolved oxygen in the composition.

Selected figure: None